DIELECTROPHORETIC CHROMATOGRAPHY WITH CROSS-FLOW INJECTION

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ABSTRACT

This paper reports the separation of particles using Dielectrophoretic (DEP) chromatography device. The device consists of a cross-flow injector, similar to that used in a capillary electrophoresis chip, and an array of interdigitated microelectrodes for separation, to which radio-frequency voltage (~5V, 1MHz) is applied. All liquid actuation is done by hydrostatic pressure. The sample is fed pulse-wise to the carrier flow using the cross-flow injector. The particles in the sample undergo the non-uniform field created by the electrode array, and more polarizable the particle is, the higher the chance of the trapping by DEP force becomes, and as a result, more delayed from the carrier flow. Thus, a separation depending on the size and the dielectric properties of the particle become possible. Using the device, the separation of 1) 0.1 μm and 1.0 μm latex particles, 2) 6.6kbp and 48.5kbp DNA, are experimentally demonstrated.

INTRODUCTION

Dielectrophoresis (DEP) refers to the motion of an electrically neutral particle resulting from the interaction between the applied electric field and induced dipole [1]. When a dielectric particle is placed in an electrostatic field, the particle polarizes, and equal amount of positive and negative charge appears, as shown in fig.1 a). In a non-uniform field, the field intensity at the positive and the negative charge is not always the same, and the resulting net force drives the motion of the particle.

The analytical expression for the DEP force $\boldsymbol{F}_{dep}$ is given by [1-2]

$$\boldsymbol{F}_{dep} = 2\pi a^3 \varepsilon_m \mathrm{Re}[K^*(\omega)]\nabla(E^2) \qquad (1)$$

$$K^*(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*},\ \varepsilon_p^* = \varepsilon_p - j\frac{\sigma_p}{\omega},\ \varepsilon_m^* = \varepsilon_m - j\frac{\sigma_m}{\omega} \qquad (2)$$

where E: field strength, a: particle radius, ε_m, ε_p: permittivities of the medium and the particle, σ_m, σ_p: conductivities of the medium and the particle, ω: angular frequency of the applied field, and j: imaginary unit.

The direction of DEP motion is towards higher field when $\mathrm{Re}[K^*(\omega)] > 0$ (particle more polarizable than the medium, Positive DEP), while the direction is towards lower field when $\mathrm{Re}[K^*(\omega)] < 0$ (particle less polarizable than the medium, Negative DEP). The determinant of the DEP force is not the charge on the particle, but the particle size and the dielectric property; its principle and performance are totally different from electrophoresis.

A remarkable property of DEP is that, as the force is proportional to E^2, it is effective in a.c. field as well as in d.c. field, so far as the polarization can follow the field alternation (fig.1 b). Because of this, DEP is particularly suitable for the actuation of biological particles in aqueous solutions. By using high-frequency field, electrochemical reactions at the electrode/solution interface can be avoided. It in turn means that the electrode can be immersed in water phase and integrated in the fluid channel. The electrode gap can be made arbitrarily small, without being annoyed by bubble generations, so that very high intensity field can be created with a moderate power supply. In fact, the voltage used in the following experiments is typically ~5V, 1MHz.

PRINCIPLE OF DEP CHROMOATOGRAPHY

The principle of DEP chromatography is shown in fig.2 [3-4]. It has a separation channel equipped with an array of microelectrodes. The carrier is flown continuously, and the sample is fed at the inlet. The particles in the sample is subjected to the non-uniform field, and one of the following occurs: i) if the particle is more polarizable than the medium, it is attracted towards electrodes by positive DEP and trapped. ii) if the particle's polarization is small, it just passes through. iii) if the particle is less polarizable than the medium, it seeks for E^2-minimum by negative DEP, and it may be pressed against the counter

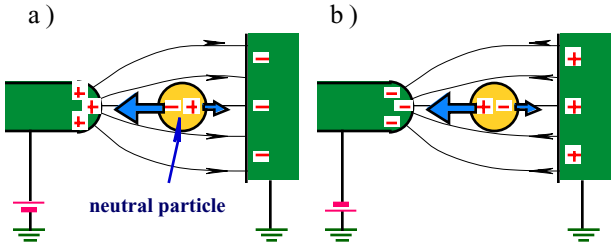

Fig.1 The Principle of Dielectrophoresis.

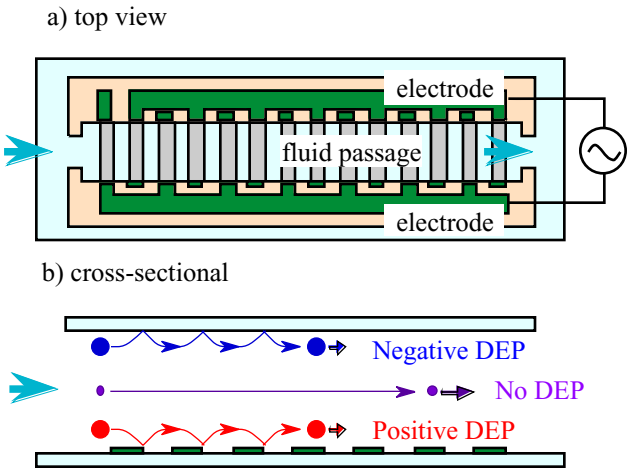

Fig.2 The Principle of DEP Chromatography

wall as shown in fig.2 b), or may be trapped at the center of the electrodes where E=0.

The trapping in both cases of positive and negative DEP is not permanent, especially for a smaller particle, where DEP force is smaller (eq.(1)) and is easily carried back to the stream by viscous drag. As a result, the particle repeats trap and release. The stronger the DEP effect, the higher the chance it is being trapped becomes, so that the particle is more delayed from the stream. Thus, a separation depending on the size and the dielectric properties of the particle become possible. The DEP chromatography can be considered as a kind of affinity chromatography, where affinity can be controlled by the magnitude and the frequency of the applied voltage.

One of the advantages of the DEP chromatography over the conventional chromatography is its low pressure drop. The column is just a hollow channel having thin-film electrodes on one side. Pumping power is low, and fast separation can be expected.

CONSTRUCTION OF THE DEVICE

The DEP chromatography device we have fabricated is schematically depicted in fig.3. It has the carrier inlet, which diverges into 8 parallel channels for separation, and converges to the outlet. Perpendicular to the main stream is the sample inlet and sample outlet, which constitute cross-flow injector. The reason why there are 8 channels in parallel is to investigate the reproducibility. There are two pairs of interdigitated electrodes, covering 4 channels each. One electrode pair is energized for DEP separation, and the other is not energized for reference. All liquid actuation is done by hydrostatic pressure. In capillary electrophoresis chip (CEP), electroosmotic flow is used for liquid actuation, but it cannot be used in the device, because the electrodes are in the channel, and application of d.c. voltage in the liquid causes electrolysis on the electrodes.

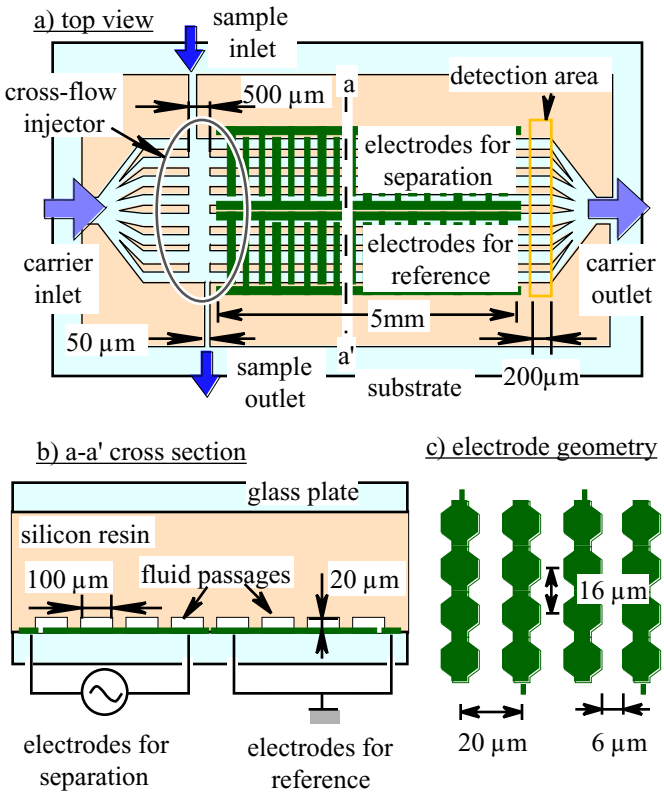

Fig.3 The DEP Chromatography Device

The enlarged view of the electrode array is shown in fig.3 c). It is made of aluminum and patterned on a glass substrate using a standard vacuum-evaporation process and photolithographic techniques. The electrode has corrugated shape as illustrated in the figure to create highly non-uniform field, with the minimum gap of 6 µm. Each electrode array has 250 electrodes arranged with 20 µm pitch, and hence the total length along the fluid channel is 5mm. To prevent adsorption of the particles, the electrode surface is covered by a thin organic layer of several nm in thickness, by spin-coating 40 times diluted negative photo-resist (CMS-EX, Tosoh, Japan).

The fluid channel is made of PDMS (polydimethylsiloxane, silicon resin KE-106, Shin-Etsu, Japan). A 20 µm thickness photo-resist film is attached on a substrate, the channel shape is patterned, PDMS pre-polymer is dropped, and covered by a glass plate. After polymerization, PDMS is peeled off, and pasted on a glass substrate having the electrode arrays. Finally a glass back plate is mounted on the other side, which prevents the deformation of PDMS when pressure is applied to actuate the liquid. The depth of the channel is determined by the photo-resist film, and is 20 µm everywhere. The dimensions of the channels are as follows: width of the 8 separation channels is 100 µm each (fig.2 b), the width of injection channel is 50µm, area of the intersection of the cross-flow injector is 1500 µm x 500 µm.

As a conclusion to the presented test the encountered tolerance of the flow as a function of actuation voltage was not found to influence the operation of the system since a sufficient flow was achieved to ascertain the pens function. In detail the flow between cartridge and nip-buffer exceeded that from the buffer to the paper. Should the system need to be adapted to an application requiring greater precision an additional flow-rate detection would be required.

A second important measurement conducted in the system design process involved the rigidity of the complete setup. Here the influence of the curvature of the housing, resulting from an external bending momentum applied across the full stretch of the pen, as e.g. provided by the operators handling of the pen, was investigated. Here particularly the housing deflection, i.e. the actual bending of the pen as compared to its normal position, was of importance. Bending of the pen causes a relative motion of the valve against the valve seat influencing its leakage. A significant influence on the flow rate was found as illustrated in figure 9. The deflection of the housing was found to strongly influence the deflection of the valve. This was to be expected as the actuator deflection of around 50µm is small as compared to the possible deflection of the housing of around 100µm. As a result of this the stiffness of the housing across the actuator was enhanced by the use of a steel collar around the appropriate section of the pen. The results are shown in figure 9 and indicate an important improvement as well as the necessity of this measure.
An important conclusion derivable from this finding is the need for adequate mechanical measures or in

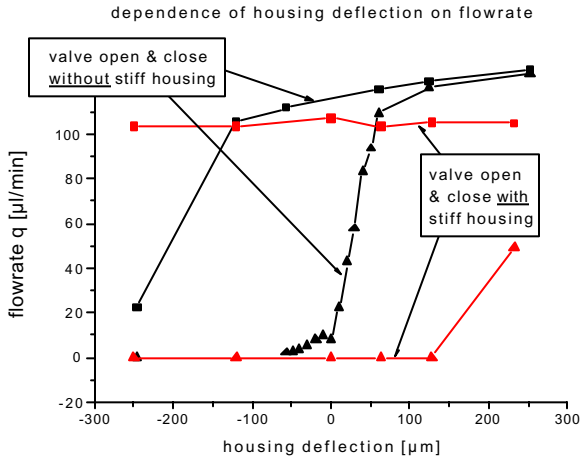

Fig. 9: Test of microvalve in different housings

consequence the need for a more deflection-tolerant actor. Here an actuator deflection of several hundred micro-meters would be advantageous.

CONCLUSIONS

A fully operable closed loop micro-dosing system is presented employing a wide range of technologies for its individual components.
The micro-machined liquid level sensor is based on conductive and capacitive detection of the ink (figure 2).
A revised piezoelectrically driven microvalve is fabricated by injection molding. It contains a highly elastic membrane resistant against a wide range of mechanical loads. Maximum leak rates of 2nl/min at 1 kPa are attained. The complete system is particle tolerant for particle diameters below 10 µm and resistant against all types of inks (pH 1–9).
The most important conclusion to be drawn from the presented project is the need for flexibility in the approaches. Also the importance of the packaging issues and the system integration has to be underlined. Specifically in micro-fluidic systems this aspect must not be underestimated as a great number of problems derive not from the individual components but from the complex interaction between them. Assuming an adequate talent single elements may be designed to work as desired. The combination of two or more of these components does not necessarily result in a functional product.
In the course of this project we have shown that not only the single elements in the system need careful consideration but that the complex interactions between them must be accounted for. For this reason the presented system design was carried out under consideration of all involved components and functions. This resulted in an integrated design of mutually compatible elements namely a fluid supply cartridge, a valve, a number of channels, a fluid level sensor, and fluid buffer compartments in a vibration tolerant setup.
The complete system was designed for a mass market consumer product, an accordingly solid construction was made.

ACKNOWLEDGEMENT

The authors wish to thank the project partners for enabling them to work on the project. The project MIKRODOS is funded by the German Federal Ministry of Research and Technology BMBF under 16SV736.

DEP SEPARATION OF DNA

Separation of DNA with the device is shown using ColE1 DNA (6.6kbp, kbp = kilo-base-pairs) and λ-DNA (48.5kbp). Both are purchased from Nippon Gene, Japan, and fluorescence-labeled with the dye YO-YO-1 (Molecular Probe Inc.). The concentrations used in the experiments are [ColE1 DNA] = 0.050 μg/μl and [λ-DNA] = 0.026 μg/μl, which are chosen so that both samples emit equal fluorescence per volume.

In fig.6 is shown the superposition of three independent measurements, 6.6kbp DNA at 0V, 6.6kbp DNA at 5V, and 48 kbp DNA at 5V. The medium is 2mM Tris-HCl + 0.2mM EDTA, whose conductivity is 0.25 mS/cm.

When no voltage is applied, the steep-rise slow-decay waveform is obtained, with the peak approximately at the fluid residence time in the channel. When 5 V is applied, the peak for 6.6 kbp DNA appears at 100 sec and 48.5 kbp DNA at 155 sec. This is in agreement with the theoretical prediction that larger particles receive larger DEP force.

Then the mixture of the two DNA samples is fed and the separation is examined. The result is shown in fig.7. Two separate peaks at 110 and 150 sec clearly appear, corresponds respectively to 6.6 and 48.5 kbp DNA. From the waveforms, the number of theoretical plate is estimated to be about 500.

In fig.7 is also plotted, with a thinner line, the chromatogram of the DNA's when the medium concentration is doubled and the conductivity is increased to 0.45 mS/cm. Under this condition, the peak for 6.6kbp DNA appears at 55 sec (~ τ_r), indicating that DEP is not effective at all, and the peak for 48 kbp DNA is at 95 sec, indicating the DEP force is reduced compared with the lower conductivity case. This tendency is also in agreement with the theoretical prediction of eq.(1): for a particle showing positive DEP, its polarizability relative to that of the medium becomes smaller as the medium conductivity is increased, so that positive DEP becomes weaker.

Positive DEP is often observed with biological particles, such as cells, DNA, proteins, organelle. Therefore, lowering the medium conductivity is often effective in having better separation in the DEP chromatography. For this purpose, we are developing a process to integrate on-chip dialysis system [5].

The results obtained in this paper suggest a promising application of DEP chromatography for the analysis of large (>kbp) DNA, which is presently done by the pulse field electrophoresis and takes hours.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Tomohisa Kawabata of Wako Pure Chemical Industries Ltd. and Mr. Hitoshi Suzuki of Advance Co. for valuable discussion. This work is in part supported by the Ministry of Education (Kakenhi No.12555076).

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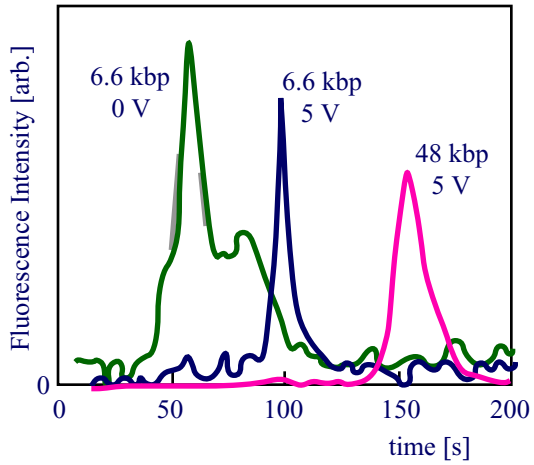

Fig.6 DEP Chromatogram for DNA with Different Size
Medium : 2mM Tris-HCl + 0.2mM EDTA, 0.25 mS/cm

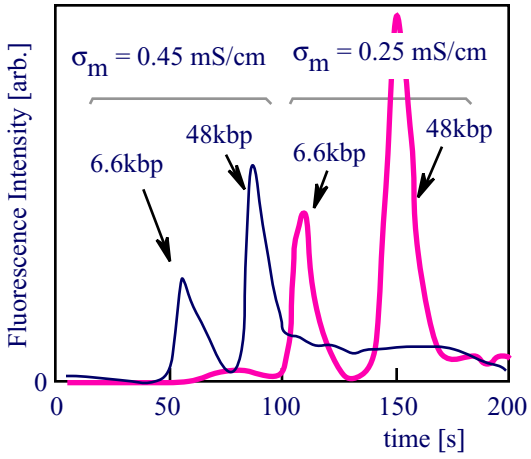

Fig.7 Separation of DNA
and the Effect of Medium Conductivity